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THE TESTING AND STANDARDIZATION OF *B. WELCHII* (*PERFRINGENS*) ANTITOXIN.

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THE recent increase in the use of *B. welchii* (*B. perfringens*) antitoxin in intestinal surgery (Williams, 1926), puerperal sepsis following abortion (Nürnberger, 1925, Lehman, 1926, and Inkster and Okell, 1924), and infection of the gall-bladder (Gould and Whitby, 1927), has directed attention to the problem of standardization of the antitoxin.

METHODS OF TESTING.

It is not proposed here to review all the references to the demonstration of antitoxic power of *B. welchii* serum, or to deal with testing against living cultures, one of the methods used by Weinberg and others. In Table I is given a summary of the chief methods of testing toxin and antitoxin used in recent years. Bull (1917) used for the titration of serum intramuscular injection of pigeons. He chose first as the "test dose" one minimal lethal dose of the filtrate of a young broth culture of *B. welchii*. His provisional "unit" of antitoxin was the smallest amount which when mixed with the test-dose of toxin and injected completely protected the pigeon against the toxin.

At the Wellcome Physiological Research Laboratories (Medical Research Committee, 1919) the intramuscular injection into mice was used, and, of the toxins available at first, the test-dose was of necessity limited by volume to two fatal doses. It was found that one-half of the Bull unit protected against two fatal doses of the chosen toxin. The W.P.R.L. unit used provisionally in the war was therefore made equivalent to twice the amount of serum that would protect against two fatal doses of the toxin originally chosen. A batch of serum was chosen as a standard and titrated as closely as possible against this original toxin. Subsequent toxins were in their turn standardized against this standard serum, but only those toxins were retained for test purposes which contained at least two fatal doses in the test-dose.

Bengtson (1920) chose as the test-dose of toxin ten times the minimum dose lethal for the pigeon when given intramuscularly; this test-dose was contained in a volume of 2 c.c. to 2·5 c.c. of the toxin then available.

Intravenous injection into rabbits and mice has been practised by many workers in this field. It was used on the large scale for testing at these laboratories during the war; the method has been developed during the past

TABLE I.—*Methods of Testing Toxin.*

Pigeon	Intramuscular	M.L.D.	0·1 to 1·0 c.c.	Bull, 1917; Bengtson, 1920.
Mouse	,,	,,	,, ,,	Present authors, 1918–1928 (*M.R.C. Report*, 1919).
,,	Intravenous	,,	0·01 to 0·1 c.c.	Ditto.
Guinea-pig	Intracutaneous	M.R.D.	,, ,,	Klose, 1916; Glenny and Allen, 1921.
In vitro	Hæmolysin	M.H.D.	,, ,,	Henry, 1917; Weinberg, 1918.

TABLE II.—*Titration of Standard Serum Against Fixed Doses of Toxin.*

Dose of standard serum.	*In vitro.*	Mouse.		Guinea-pig.
	Hæmolysis.	Intra-venous.	Intra-muscular.	Intradermic.
0·0018	Complete	...	Died ,,	... Necrosis.
0·0020	,,	Died ,,	Œdema Lived	Slight necrosis.
0·0022	Nearly complete	,, Lived	,, ,,	No necrosis.
0·0025	Slight hæmolysis	,, ,,	,, ,,	...
0·003	No hæmolysis	,, ,,	,, ,,	...

TABLE III.—*Neutral Points.*

Standard serum	0·0025	0·0022	0·0020	0·002
Serum A	0·014	0·012	0·01	0·012
,, B	0·00065	0·0006	0·0006	0·00055–75
,, C	0·0021	0·002	0·0017	0·0016
,, D	0·001	0·00075	0·0007	0·0008

Calculated Values.

(Standard = 45 units.)

Serum A	8	8	9	8
,, B	170	160	150	120–170
,, C	50	50	50	60
,, D	110	130	130	120

few years and much used by Dalling and Mason (1924), first in connection with "lamb dysentery cultures" (? *B. welchii*), and later with *B. welchii* (1926).

The intracutaneous method of testing in guinea-pigs mentioned by Klose (1916) was used by Glenny and Allen (1921), and has been much used during the present work. The technique of this method differs in no essential detail from that practised in testing diphtheria toxin and antitoxin; the lesion is necrotic, appears in twenty-four hours and quickly heals, but can be traced for some days later.

It is important to know to what extent the results of these methods of titration agree. Towards the end of the war, Glenny and Allen traced "primary and secondary response" of horses to injections of *B. welchii* toxin or toxin-antitoxin mixtures, using simultaneously for titration the intramuscular injection of mice and intracutaneous injection of guinea-pigs. The results given by the two methods showed fairly close agreement. About the same time in a limited number of experiments they found a close relation between the results of the intravenous and intramuscular methods of titration. This comparison of methods has been taken up again recently. A typical result is shown in Table II. It will be seen that there is a close agreement between the values given by the three methods. We have appended Table III (*v. infra* units of measurement), in which are shown the values of four different sera. For convenience a fixed dose of test toxin was used and the necessary calculations made for the intradermic method.

A considerable amount of further work will be necessary with numbers of different toxins from different strains and different antitoxins before it can be stated that the agreement holds under all circumstances; this and an inquiry into the toxin and toxoid relationships are in progress and the results will be published later.

In Table II is also shown the result of titration of the anti-hæmolytic property of the antitoxin. Throughout the literature much attention has been directed to the hæmolytic powers of *B. welchii* and its toxin. In recent years Weinberg (1918) and Henry (1922) have examined and titrated hæmolysin. Dalling, in his lamb dysentery work, investigated the relation of the hæmolysins and anti-hæmolysins connected with "lamb dysentery cultures" and *B. welchii*. More recently Mason, Glenny and Dalling (in preparation for publication) have found that there is apparently the close relationship shown in the table.

Our colleagues, Buttle and Trevan, kindly permit us to say that they have been investigating for some time past methods of "physiological standardization," using excised smooth muscle-tissue suspended in Ringer solution in the examination of toxins of the gas-gangrene anaerobes.

UNITS OF MEASUREMENT.

The provisional war units already referred to, established as they were against one or two fatal doses of toxin, served their purpose at the time. For the purpose of fixing a unit it would on general grounds be desirable to choose a test-dose of *B. welchii* toxin containing approximately 100 minimum lethal doses if this amount were contained in a convenient volume of the toxin, as in the case of diphtheria, tetanus, *botulinus* and *œdematiens* toxins. The volume of 100 m.l.d. of *B. welchii* toxin would be inconveniently large; it is, however, possible to make use of 10 m.l.d. in the pigeon-intramuscular method and the mouse-intravenous method.

A toxin having been chosen whose test-dose is contained in a convenient volume and representing a reasonable number of lethal doses, it is next necessary to find its equivalent in standard serum; this now becomes the standard to which all future references are made. Whether this equivalent is termed one unit or some fraction of a unit is a matter for later consideration.

Bengtson determined the amount of a certain serum protecting against ten pigeon-intramuscular fatal doses of a certain toxin and called it 1/100th of a unit. The standard serum chosen by her contained 100 equivalents, and therefore 1 unit per c.c.

The method chosen by us was as follows:

A "toxin" was prepared, precipitated by ammonium sulphate, dried and ground; this material is reasonably stable, whereas the liquid toxin may rapidly deteriorate. A typical titration of such a toxin is shown in Table IV. Approximately ten times the mouse-intravenous fatal dose was accepted as a convenient "test dose" of toxin. A certain serum of low value was chosen as our standard serum. The volume of this serum which, when mixed with the test dose of toxin and injected into mice, protected half of a group of six or more mice, was determined in a series of tests; it proved to be 0·0022 c.c. A dose of serum about 10% higher, 0·0025 c.c., usually protected 90% or more of the mice, whereas a dose 10% lower, 0·0020 c.c., usually failed to protect 90% or more of the mice.

TABLE IV.—*Titration of Toxin.*

c.c. of toxin. 16 mgm. per c.c.	Precipitated : Mice, intravenously.		
0·03	+	+	+
0·025	+	+	L
0·02	+	+	L
0·015	L	L	L
0·01	L	L	

A second test toxin was titrated against the standard serum until a test dose was chosen which gave corresponding results with these doses of the test serum. This test dose of toxin was found to contain nearly 20 fatal doses. Thus it is seen that, as in the case of diphtheria and tetanus and other toxins, the number of fatal doses contained in the "test dose" of toxin is not constant, but depends on the relation between the amount of toxin and toxoid present.

Possibly the English Authority under the Therapeutic Substances Act, the U.S.A. Public Health Service and the League of Nations Standardization Committee will eventually decide on an official unit. Meanwhile we felt that it was advisable to choose a unit of antitoxin such that the strength of an antitoxin expressed in "units" would be of the same order as that of the well-established sera such as those of diphtheria and tetanus. We considered, therefore, adopting as our unit ten times the amount of serum that protects against the originally chosen "test dose" of toxin; this would be contained in 0·022 c.c. of our standard serum. When we tested by the mouse-intravenous method a sample of the stock of antitoxin held at the Hygienic Laboratory, Washington, which Dr. McCoy kindly sent to us, we found that one "Bengtson unit" was equivalent to 120 of our units. To avoid numerical complication we are now altering our unit so that the relation between the "Bengtson unit" and the provisional unit we are adopting (the "W unit") is exactly 100 to 1, *i.e.* 0·01 c.c. of the Hygienic Laboratory serum is equal to our "W unit."

If the unit suggested by Bengston for *B. welchii* antitoxin were adopted, serum laboratories with horses producing many different antitoxins would find that the serum obtained from horses of high value would contain, in the case of diphtheria, 2000 units and upwards, for tetanus 2000 units and upwards, for *B. welchii*, on the other hand, 3 to 5 units; there would be a corresponding lack of relation in the figures used by the clinician in deciding on dosage. We feel that it would be of advantage to maintain as close a relation as practicable between the "dosage figures" which the clinician has to remember in prescribing treatment with sera. We therefore suggest that a unit 100 times smaller than the "Bengtson unit" would be the most convenient to use. There would appear to be no very sound reason why units of all antitoxin should be expressed in numbers containing many cyphers, and probably the "thousands" in the nomenclature of units of sera could be dropped with advantage, but if such a change is to be made, we would suggest that it would be advisable to make the change for all sera at one time. In Table V we have summarized the important points relating to the units of measurement.

TABLE V.—*Units of Measurement.*

			Minimal number of M.L.D. in test-dose.	Number of test-doses neutralized by one unit.	Expressed in "W" units.
Temporary war units :					
Bull	Pigeon	Intramuscular	1	1	$\frac{1}{10}$ to $\frac{1}{20}$
W.P.R.L.	Mouse	,,	2	2	
Modern units :					
Bengtson	Pigeon	Intramuscular	10	100	100
W.P.R.L.	Mouse	Intravenous	10	10	1

SUMMARY AND CONCLUSIONS.

1. *B. welchii* antitoxin has been titrated against precipitated toxin by intramuscular or intravenous injection into mice, intradermic injections into guinea-pigs, determination of anti-hæmolytic titre *in vitro*, or by means of excised smooth muscle-tissue.

2. All these methods are probably but different means of titrating the same principle since the values given agree fairly closely.

3. A provincial "unit" of antitoxin corresponding to 0·01 c.c. of the "Bengtson unit" serum has been adopted.

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